

What is claimed is:

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1. An imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin.
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2. A composition comprising an effective imaging amount of the imaging agent of claim 1 and a physiologically acceptable carrier.
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3. An agent of claim 1, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.
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4. An agent of claim 3, wherein the marker is a radioactive isotope.
5. An agent of claim 4, wherein the radioactive isotope is indium-111.
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6. An agent of claim 4, wherein the radioactive isotope is technetium-99m.
7. An agent of claim 4, wherein the radioactive isotope is iodine-123, iodine-125, iodine-131, krypton-81m, xenon-133, or gallium-67.
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8. An agent of claim 1, wherein the polypeptide is a 31 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of

human fibronectin and having th amino acid sequence of amino acids 1-262 as shown in Figure 1.

- 5 9. An agent of claim 1, wherein the polypeptide comprises a 20 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-153 as shown in Figure 1.
- 10 10. An agent of claim 9, wherein the polypeptide comprises less than about 20 additional amino acids.
- 15 11. An agent of claim 1, wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-109 as shown in Figure 1.
- 20 12. A method for imaging a fibrin-containing substance which comprises contacting the fibrin-containing substance to be imaged with the agent of claim 1 under conditions such that the agent binds to the  
25 fibrin-containing substance and imaging bound agent and thereby imaging the fibrin-containing substance.
- 30 13. A method of claim 12, wherein the fibrin-containing substance is a thrombus.
14. A method of claim 12, wherein the fibrin-containing substance is atherosclerotic plaque.

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15. A method for imaging a fibrin-containing substance in a subject which comprises:

(a) administering to the subject a composition of claim 2 under conditions permitting the imaging agent contained therein to enter the blood stream and bind to fibrin present in the blood vessels;

(b) imaging bound agent within the blood vessels; and thereby

(c) imaging the fibrin-containing substance.

16. A method of claim 15, wherein the fibrin-containing substance is a thrombus.

17. A method of claim 15, wherein the fibrin-containing substance is atherosclerotic plaque.

18. A method of claim 12, wherein the polypeptide is a 31 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-262 as shown in Figure 1.

19. A method of claim 12, wherein the polypeptide comprises a 20 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-153 as shown in Figure 1.

20. A method of claim 19, wherein the polypeptide comprises less than about 20 additional amino acids.
21. A method of claim 12, wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-109 as shown in Figure 1.
22. A method of claim 12, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.
23. A method of claim 12, wherein the imaging is carried out using a gamma camera.
24. A plasmid for expression of a polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin comprising DNA encoding the polypeptide and DNA encoding suitable regulatory elements positioned relative to the DNA encoding the polypeptide so as to effect expression of the polypeptide in a suitable host cell.
25. A plasmid of claim 24, wherein the polypeptide is about a 31 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin.
26. A plasmid of claim 24, wherein the polypeptide comprises about a 20 kD polypeptide corresponding

to an amino acid sequence present in the fibrin binding domain of human fibronectin.

- 5 27. A plasmid of claim 24, wherein the polypeptide is about a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin.
- 10 28. A plasmid of claim 25, wherein the polypeptide is a 31 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-262 as shown in Figure 1.
- 15 29. A plasmid of claim 26, wherein the polypeptide comprises a 20 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-153 as shown in Figure 1.
- 20 30. A plasmid of claim 29, wherein the polypeptide comprises less than about 20 additional amino acids.
- 25 31. A plasmid of claim 27, wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-109 as shown in Figure 1.
- 30 32. A plasmid according to claim 28 designated pFN 975-25 and deposited in Escherichia coli strain A4255 (F<sup>+</sup>) under ATCC Accession N . 67832.
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33. A plasmid according to claim 30 designated pFN 949-2 and deposited in Escherichia coli strain A1645 under ATCC Accession No. 67831.
- 5 34. A plasmid according to claim 31 designated pFN 196-2 and deposited in Escherichia coli strain A4255 under ATCC Accession No. \_\_\_\_.
- 10 35. A cell which comprises the plasmid of claim 24.
36. A bacterial cell according to claim 35.
37. An Escherichia coli cell according to claim 36.
- 15 38. An Escherichia coli cell according to claim 37, wherein the plasmid is the plasmid designated pFN 975-25 and wherein the cell is deposited under ATCC Accession No. 67832.
- 20 39. An Escherichia coli cell according to claim 37, wherein the plasmid is designated pFN 949-2 and wherein the cell is deposited under ATCC Accession No. 67831.
- 25 40. An Escherichia coli cell according to claim 37, wherein the plasmid is designated pFN 196-2 and wherein the cell is deposited under ATCC Accession No. \_\_\_\_.
- 30 41. A method of producing a polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin which comprises treating the cell according to
- 35 claim 35 so that the DNA directs expression of the

polypeptid and the cell express s the polypeptide and recovering from the cell the polypeptide so expressed.

- 5 42. A method of producing a 31 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin which comprises treating an Escherichia coli cell comprising the plasmid of claim 28 so that the DNA directs expression of the polypeptide and the cell expresses the polypeptide and recovering from the cell the polypeptide so expressed.
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- 15 43. A method of producing a 20 kD polypeptide fragment corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin which comprises treating an Escherichia coli cell comprising the plasmid of claim 29 so that the DNA directs expression of the polypeptide and the cell expresses the polypeptide and recovering from the cell the polypeptide so expressed.
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- 25 44. A method of producing a 12 kD polypeptide fragment corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin which comprises treating an Escherichia coli cell comprising the plasmid of claim 31 so that the DNA directs expression of the polypeptide and the cell expresses the polypeptide and recovering from the cell the polypeptide so expressed.
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- 5 45. A purified polypeptid substantially free of other substances of human origin which has an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin.
- 10 46. A polypeptide of claim 45, wherein the polypeptide is a 31 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-262 as shown in Figure 1.
- 15 47. A polypeptide of claim 45, wherein the polypeptide comprises a 20 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-153 as shown in Figure 1.
- 20 48. A polypeptide of claim 47, wherein the polypeptide comprises less than about 20 additional amino acids.
- 25 49. A polypeptide of claim 45, wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of human fibronectin and having the amino acid sequence of amino acids 1-109 as shown in Figure 1.
- 30 50. A polypeptide of claim 45 fused to a second polypeptide which comprises a substantial portion of the amino acid sequence of the cell binding domain of naturally-occurring human fibronectin.



51. A 45 kD fused polypeptid of claim 50, wherein the polypeptide is a 12 kD polypeptide and the second polypeptide is a 33 kD polypeptide.
- 5 52. A polypeptide of claim 45, wherein the amino acid sequence GRGDS is fused to the N-terminus of the 31 kD polypeptide.
- 10 53. A 64 kD fused polypeptide of claim 50, wherein the polypeptide is a 31 kD polypeptide and the second polypeptide is a 33 kD polypeptide.
- 15 54. A plasmid for expression of the 45 kD fused polypeptide of claim 51 designated pFN 202-5.
- 20 55. A plasmid for expression of the polypeptide of claim 52 designated pFN 195-4.
- 25 56. A plasmid for expression of the 64 kD fused polypeptide of claim 53 designated pFN 194-2.
- 30 57. A method of treating a subject susceptible to, or afflicted with, a bacterial infection which comprises administering to the subject an amount of a polypeptide of claim 45 effective to prevent or treat the bacterial infection.
- 35 58. A method of claim 57, wherein the subject is susceptible to, or afflicted with, a bacterial infection due to the presence of a catheter or an implant in the subject.
59. A coated medical device comprising a medical device and the polypeptid of claim 45 applied as a coating to the surfac f the medical d vic .

60. A coated medical device of claim 59, wherein the medical device is a catheter.

5 61. A method of minimizing risk of bacterial infection associated with use of medical devices which comprises:

(a) applying the polypeptide of claim 45 as a coating to a surface of the device; and

10 (b) employing the resulting coated device rather than an uncoated device.

15 62. A method of claim 61, wherein the medical device is a catheter.

20 63. A method of minimizing risk of bacterial infection associated with use of medical devices which comprises employing the coated device of claim 59 rather than an uncoated device.

25 64. A method of refolding and reoxidizing a polypeptide having an amino acid sequence substantially present in fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin which comprises contacting the polypeptide with a thiol-containing compound and a disulfide so as to refold and reoxidize the polypeptide.

30 65. A method of claim 64, wherein the thiol-containing compound is selected from the group consisting of glutathione, thioredoxin,  $\beta$ -mercaptoethanol, and cysteine.

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66. A method of claim 64, wherein the thiol-containing compound is  $\beta$ -mercaptoethanol and the disulfide is produced in situ by introduction of air.

5 67. A method of claim 64, wherein the polypeptide is selected from the group consisting of a 31 kD polypeptide, a 20 kD polypeptide and a 12 kD polypeptide.

10 68. A method of claim 64, which additionally comprises contacting the polypeptide with a denaturant.

69. A method of claim 68, wherein the denaturant is guanidine hydrochloride or urea.

15 70. A method of claim 64, wherein the polypeptide is at a low concentration.

20 71. A method of claim 70, wherein the concentration is below 600  $\mu\text{g/ml}$ .

25 72. A method for recovering a purified biologically active polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin from a cell in which the polypeptide has been produced by means of expression of a plasmid containing DNA encoding the polypeptide which comprises:

30 (a) disrupting the cell so as to produce a lysate containing the polypeptide;

35 (b) centrifuging the lysate so as to concentrate the polypeptide;

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- (c) separating the concentrated polypeptide ;
- (d) solubilizing the separated, concentrated polypeptide;
- (e) refolding and reoxidizing the solubilized polypeptide;
- (f) separating the refolded and reoxidized polypeptide; and
- (g) recovering the purified, refolded and reoxidized polypeptide.

73. A method of claim 72, wherein the refolding and reoxidizing comprises contacting the polypeptide with a thiol-containing compound and a disulfide so as to refold and reoxidize the polypeptide.
74. A method of claim 73, wherein the thiol-containing compound is selected from the group consisting of glutathione, thioredoxin,  $\beta$ -mercaptoethanol, and cysteine.
75. A method of claim 73, wherein the thiol-containing compound is  $\beta$ -mercaptoethanol and the disulfide is produced in situ by introduction of air.
76. A method of claim 73, wherein the polypeptide is selected from the group consisting of a 31 kD polypeptide, a 20 kD polypeptide and a 12 kD polypeptide.
77. A method of claim 73, which additionally comprises contacting the polypeptide with a denaturant.

78. A method of claim 77, wherein the denaturant is guanidine hydrochloride or urea.
79. A method of claim 73, wherein the polypeptide is at a low concentration.
80. A method of claim 79, wherein the concentration is below 600  $\mu\text{g/ml}$ .
81. A method of claim 72, wherein the separating of the concentrated polypeptide in step (c) comprises chromatography.
82. A method of claim 81, wherein the chromatography comprises Heparin-Sepharose chromatography.
83. A method of inhibiting thrombus formation in a subject susceptible to thrombus formation which comprises administering to the subject an amount of a polypeptide of claim 45 effective to inhibit thrombus formation.
84. A method of inhibiting thrombus formation in a subject susceptible to thrombus formation which comprises administering to the subject an amount of a polypeptide of claim 50 effective to inhibit thrombus formation.
85. A polypeptide in accordance with claim 45 bound to a thrombolytic agent.
86. A polypeptide bound to a thrombolytic agent in accordance with claim 85, wherein the thrombolytic agent is selected from the group consisting of: tissue plasminogen activator (TPA), urokinase,

streptokinase, prourokinas , Anisoylated  
Plasminogen-Streptokinase Activator Complex  
(Eminase™), TPA analogs, or a protease.

- 5 87. A method for achieving thrombolysis of a thrombus  
which comprises administering to a subject an  
amount of the polypeptide of claim 85 effective to  
achieve thrombolysis.

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